Determination of Acidic and Neutral Therapeutic Drugs in Human Blood by LC-ESI-MS/MS

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Introduction

In forensic toxicology, body fluids are monitored for therapeutic drugs that may have been abused, resulted in poisonings and death or impacted the ability to drive a vehicle. One family of frequently monitored drugs that are characterised by acidic or neutral chemical properties is composed of non-opioid analgesics, anticonvulsants and barbiturates.

HPLC-DAD are often used to monitor the levels of acidic and neutral therapeutic drugs in body fluids. However, by application of LC-MS/MS, a more generic and selective method may be obtained, that does not require pre-concentration and clean-up.

The present LC-MS/MS method [1] was developed in order to obtain a simple technique for screening and confirmatory analyses of live and post mortem whole blood samples.

Extraction

100 µl sample + 300 µl H_2O + 100 µl IS* + 100 µl MeOH

↓ *Mix*

+ 900 µl MeCN

↓ *Mix, 30 min stand time, 3000g/5 min*

100 µl + 400 µl H_20 in a UF cup (30 kDa cellulose membrane, 0.5 ml Amicon Ultra, Millipore)

↓ *10.000g/20 min*

LC vial

* : 1-hydroxy-2-naphthoic acid in 50% MeOH for screening
3-acetamidophenol and 1-hydroxy-2-naphthoic acid in 50% MeOH for quantification

Matrix matched calibrants based on live whole blood were prepared in the same way. The 100 µl MeOH was substituted by equivalent volumes of mixed standard solutions in MeOH.

Chromatography

Table 1
Chromatographic conditions.

	Screening	Confirmation
Column	Synergi Polar-RP column (4 µm, 2.0 mm I.D. × 50 mm)	Synergi Polar-RP (4 µm, 2.0 mm I.D. × 150 mm)
Mobile phases	A: 0.1% HAc, B: MeOH+0.1% HAc	A: 0.1% HAc, B: MeOH+0.1% HAc
Gradient	10 → 90% B over 2.5 min	10 → 90% B over 19 min
Flow rate	0.2 ml/min	0.2 ml/min
Injection volume	10 µl	10 µl
Total runtime	16 min	34 min

Mass spectrometry

Table 2
Precursor ions and transition products.

Drug	ESI ion mode	Transition	
		Q1	Q3
Diclofenac	+	296	215/250
Ibuprofen	-	205	161
Naproxen	-	229	185/170
Etodolac	-	286	242/212
Phenytoin	-	251	102/208
Lamotrigine	+	256	211/159
Carbamazepine	+	237	192/179
10-OH-carbazepine	+	255	237/194
Barbital	-	183	140/85
Pentobarbital	-	225	182/85
Amobarbital	-	225	182/85
Phenobarbital	-	231	188/85
Cyclobarbital	-	235	192/85
Paracetamol	+	152	110/93
Salicylic acid	-	137	93/65
Caffeine	+	195	110/83
3-Acetamidophenol (IS)	+	152	110
1-OH-2-Naphthoic acid (IS)	-	187	143

Instrument: Micromass Quattro Micro API triple quadrupole with an ESCi ion source.

Underscored transition products were used as quantifiers. The other transition products were used as qualifiers in the confirmatory analysis.

Amobarbital and pentobarbital could not be separated by chromatography under acidic conditions. However, sufficient separation for identification was obtained under basic condition on a Hypersil Green ENV, 3 µm, 2.1 mm I.D. × 100 mm (Thermo).

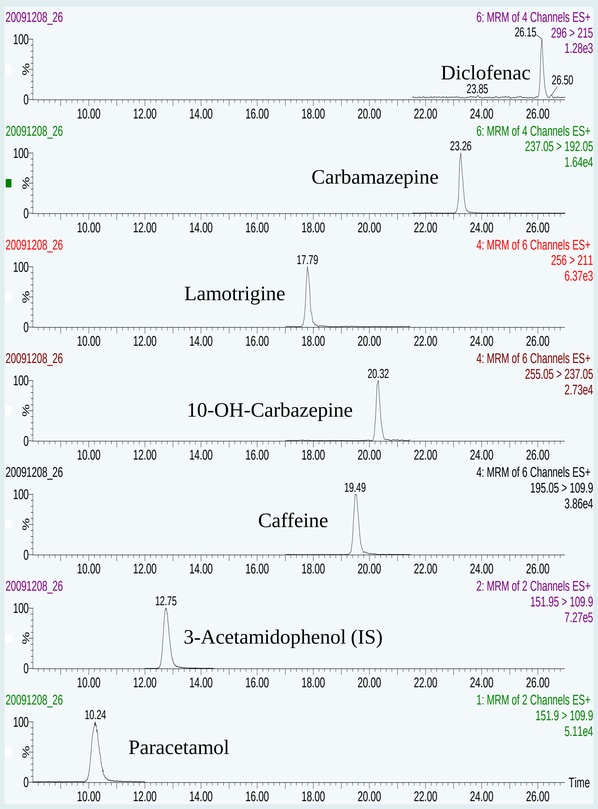

Fig. 1
ESI(+) quantifier product ions in the extract of live whole blood fortified with 1 mg/l of each substance, except paracetamol (2 mg/l) and diclofenac (0.1 mg/l).

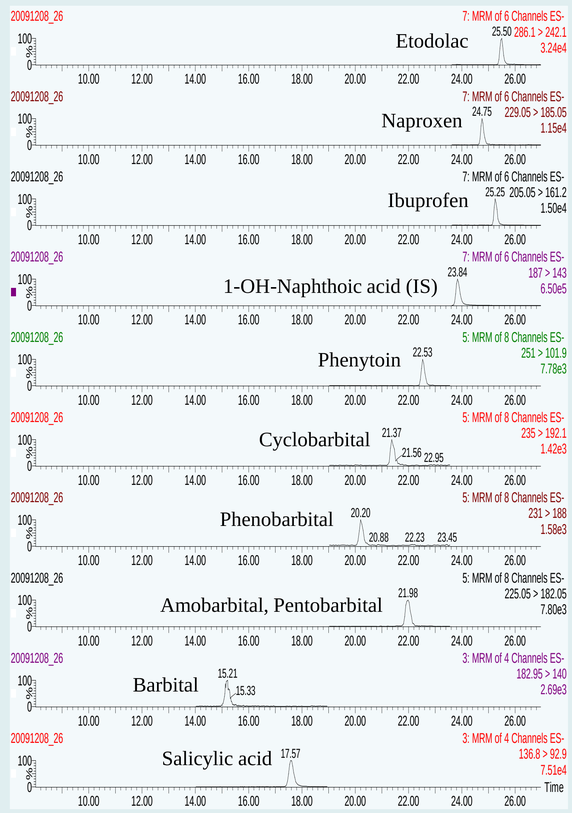

Fig. 2
ESI(-) quantifier product ions in the extract of live whole blood fortified with 1 mg/l of each substance, except salicylic acid (2 mg/l).

Method performance

The developed extraction and clean-up procedure was applied for both live and post mortem whole blood samples. The mean true recoveries were better than 92% for live blood and 84% for post-mortem blood. The mean ion suppression effects were, in most cases, less than 5% and were not significantly different for the two types of blood samples. The limits of detection (Table 3) and the precision figures (Table 4) were also not significantly different for live and post mortem blood.

Table 3
Limits of detection.

Drug	Trans. product *m/z*	LOD mg/l	Ther. conc. range [2] mg/l
Diclofenac	215/250	0.02/0.03	0.1-2.2
Ibuprofen	161	0.04	15-30
Naproxen	185/170	0.03/0.05	25-75
Etodolac	242/212	0.02/0.02	20-50
Phenytoin	102/208	0.05/0.09	8-20
Lamotrigine	211/159	0.07/0.07	2-15
Carbamazepine	192/179	0.03/0.05	4-12
10-OH-carbazepine	237/194	0.02/0.05	12-35
Barbital	140/85	0.07/0.35	5-30
Pentobarbital	182/85	0.09/0.29	1-10
Amobarbital	182/85	0.07/0.27	2-12
Phenobarbital	188/85	0.32/0.47	2-30
Cyclobarbital	192/85	0.30/0.43	2-10
Paracetamol	110/93	0.06/0.10	10-20
Salicylic acid	93/65	0.05/0.2	20-300
Caffeine	110/83	0.08/0.11	8-20

Table 4
Relative intra-lab reproducibility SDs.

Drug	Conc. level mg/l	$RSD_{R\ intra\text{-}lab}$ %
Diclofenac	0.5	8
Ibuprofen	10-40	8
Naproxen	10-40	5
Etodolac	10-40	6
Phenytoin	10-40	5
Lamotrigine	1-40	6
Carbamazepine	1-40	8
10-OH-carbazepine	10-40	5
Barbital	10-40	5
Pentobarbital	1-10	8
Amobarbital	1-10	8
Phenobarbital	1-40	8
Cyclobarbital	1-10	8
Paracetamol	10-80	4
Salicylic acid	10-80	5
Caffeine	10-40	6

Conclusion

A simple, rugged and sensitive LC-MS/MS method was developed and validated for the simultaneous determination of fifteen therapeutic drugs with acidic or neutral chemical properties in live and post-mortem whole blood. The substances included in the method validation were selected analgesics, anticonvulsants and barbiturates, which are frequently monitored in forensic samples. The developed method would be applicable as a generic analytical technique for this family of drugs.

[1] L.K. Sørensen, Determination of acidic and neutral therapeutic drugs in human blood by liquid chromatography-electrospray tandem mass spectrometry, Forensic Sci. Int. *In press*. Online DOI:10.1016/j.forsciint.2010.07.016

[2] The International Association of Forensic Toxicologists (TIAFT), http:/www.tiaft.org.

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